

REMARKS ON THE CHROMOSOME NUMBERS IN THE RUBIACEAE

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INTRODUCTION

Although the Rubiaceae is one of the earliest families recognized by many botanists, clarification of relationships between related families and of taxa within the family remains to be completed.

Rubiaceae as considered by Cronquist (1968, 1981) is somewhat aberrant family, in which the evaluation of multi-disciplinary data will likely bring many changes. Earlier, the order Rubiales (of both Bessey and Engler, Rubicinae of Hallier) was composed of Rubiaceae, Adoxaceae, Valerianaceae, Caprifoliaceae, and Dipsacaceae. Bentham and Hooker (1862-1883) retained only Caprifoliaceae including Adoxaceae, and Rubiaceae in the Rubiales and transferred the rest to the Asterales. Hutchinson (1959) retained only Rubiaceae in the Rubiales and transferred Caprifoliaceae to the Araliales (Archechlamydeae), Adoxaceae to Saxifragales, Valerianaceae and Dipsacaceae to Valerianales.

More recently Wagenitz (1959) indicated that the order Rubiales as defined in Engler's Syllabus cannot be regarded as natural, because the similarities between Rubiaceae and other families included in this order appear to be less important than those between Rubiaceae and some of the families that were included in the order designated Contortae. Wagenitz (1959), Thorne (1983), and Takhtajan (1980) transferred Rubiaceae to the order Gentianales, and indicated a relationship to the family Loganiaceae. The other families, of the former Rubiales, are maintained in the order Dipsacales as described by Lindley (emended by Nakai). Cronquist (1968, 1981) considered it useful to maintain the single family Rubiaceae in the Rubiales.

Although relatively little is known cytologically, comparing to size of family and economic importance of it, a reasonable amount of data have accumulated in the last 40 years. Fagerand published a comprehensive report in 1939 which allowed him to determinate chromosomal inter-relationships and phylogenetic consideration of the family. Chromosome information has been used to indicate some unsuspected relationships and/or endorsed some existing opinions concerning possible affinities. Many gaps presently exist in the chromosomal knowledge, and some interpretations may be changed as additional cytological data are accumulated. Nevertheless, it should be indicated that chromosomal information would be one of the important aspects to better understanding this family.

In this publication, the base haploid number, polyploidy, distribution, ancestry and evolution regarding chromosome number will be discussed.

METHODS

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Chromosome numbers were compiled for the Rubiaceae from the literature. Major references of Rubiaceae and related families were: Chromosome Numbers of Flowering Plants (in Russian) by Bolkhovskikh et al. (1966), Index to Plant Chromosome Numbers 1967-71, 1972 by Moore (1973, 1974), and IOPB Chromosome Number Report (1973-79) in Taxon. Some of earlier and apparently erroneous counts have been omitted. The arrangement of genera into tribes follows Wagenitz (1964). The related families were arranged following Takhtajan's classification system (1980). Data on geographic distribution has been compiled mainly from Wagenitz (1959, 1964), Verdcourt (1959), and specimens in the New York Botanical Garden Herbarium.

RESULTS and DISCUSSION

Rubiaceae like most of the large dicotyledonous families have received comparatively little cytological attention. The chromosome numbers of 730 species were collected. These include little more than 10 % of the whole family. However, taxa from apparently all the grouping have been included. Thus this permits some reasonable consideration regarding base number, polyploidy, distribution, inter-/intra-relationships, ancestry and evolution of the family.

A spectrum of chromosome numbers is known to exist in the family between the lowest $2n=12$ (Hedyotis nutalliana Fosb., H. watsonii Lewis), and highest $2n=220$ (Coprosma ernodeoides A. Gray, Galium grande McClatchie). Other taxa with very high numbers are: Coprosma pumila Hook f. ($2n=154$), Galium ovata (Wawra) Skottsb. ($2n=102, 104$), G. palustre L. var. aparinoides Neum ($2n=100$), Kadua centranthoides Hook et Arn. ($2n=100$), and Galium elongatum Presl. ($2n=96$).

The base numbers of the genera reveal the presence of 6 through 14, and of 17 series. The predominant base number in the family is $x=11$ (2, 3, 6, 8, 10, 12, 14, $20x$) followed by $x=9$ (4, 6, $8x$) (Table 1). A majority of the known chromosome numbers from diverse tribes have a base number of $x=11$. A high degree of morphological differentiation has taken place in the family without a large number of changes in chromosome numbers (Stebbins 1950). The base number $x=9$ could be of independent origin or secondarily derived from $x=11$. It is possible that those close to base number $x=11$ might have been derived by aneuploidy, with addition ($x=12$) such as Catesbaea latifolia Lindbl., or reduction ($x=10$) such as Asperula molluginoides Reichenb.

Some authors have indicated the possible relationships of Rubiaceae with other families. Apart from morphological similarities, researchers should determine families which have genera predominantly based on $x=11$ and its derivatives. Rubiaceae seemingly form a connecting link between the Gentianales and Dipsacales, and yet they would be an abnormal element in either order (Cronquist 1968). The characteristics of the groups have been discussed by Wagenitz (1959), who concluded that Rubiaceae should be included in the Gentianales, and resemblance of Rubiaceae to Caprifoliaceae (Dipsacales) is the result of convergence rather than indication of close relationship. The evidence from considering its predominant base numbers supports Wagenitz's opinion. In the Gentianales, Rubiaceae is more similar

Table 1. Distribution of chromosome numbers in tribes of Rubiaceae. An: aneuploidy. Bremekamp's system is followed. See the text for the Group "A" and "B".

| Chromosome Number | Tribe | Anthospermeae | Chiococceae | Cinchoneae | Gardenieae | Guettardeae | Hamelieae | Hedyotideae | Ixoreae | Knoxieae | Morindeae | Mussaendeae | Nauclaeae | Paedericeae | Psychotrieae | Rondeletieae | Rubieae | Spermacoceae | Vanguerieae | Unknown | Total |
|-------------------|-------|---------------|-------------|------------|------------|-------------|-----------|-------------|---------|----------|-----------|-------------|-----------|-------------|--------------|--------------|---------|--------------|-------------|---------|-------|
| 12 (2n) | | | | | | | | 5 | | | | | | | | | | | | | 5 |
| 14 | | | | | | | | 1 | | | | | | | | | | | | | 1 |
| 16 | | | | | | | | 3 | | | | | | | | | | | | | 3 |
| 18 | "A" | | | | | | | 25 | | | | 2 | | | | | 4 | | | 7 | 38 |
| 20 | | | | | | | | 8 | | 1 | | | | | | | | | | 6 | 15 |
| 22 | | 8 | | 2 | 20 | | | 13 | 55 | | 2 | 9 | 1 | | 23 | 3 | 122 | 1 | | 34 | 293 |
| 24 | | | 1 | | | | 3 | 3 | | | | | | | | | 8 | | | | 15 |
| 26 | | | | 1 | | | | 5 | | | | | | | | | | | | | 6 |
| 28 | | | | | | | | 2 | | | | | | | | | | 13 | | | 15 |
| 32 | | 1 | | | | | | 5 | | | | | | | | | | | | | 6 |
| 33 | | | | | | | | | 1 | | | | | | | | | | | | 1 |
| 34 | | | | 3 | 2 | | | | | | | | | | | | | | | | 5 |
| 36 | "B" | | | 6 | | | | 17 | | | | 2 | | | | | | | | 11 | 36 |
| 40 | | 1 | | 3 | | | | 1 | | 6 | | | | | | | | | | 3 | 14 |
| 44 | | 12 | 1 | | | 2 | | 1 | 11 | | | 1 | 4 | 1 | 8 | 2 | 96 | 1 | 5 | 10 | 155 |
| 48 | | | | | | | 5 | 2 | | | | | | | | | 3 | | | | 10 |
| 54 | | | | | | | | 2 | | | | | | | | | | | | | 2 |
| 56 | | | | | | | | | | | | | | | | | | 4 | | | 4 |
| 66 | | 1 | | | | | | | 2 | | | | | | | | 20 | | | 1 | 24 |
| 68 | | | | 3 | | | | | | | | | | | | | | | | | 3 |
| 72 | | | | 6 | | | | 3 | | | | | | | | | | | | 1 | 10 |
| 84 | | | | | | | | | | | | | | | | | | 4 | | | 4 |
| 88 | | | | | | | | | 1 | | | | | | 1 | | 11 | | | | 13 |
| 96 | | | | | | | | | | | | | | | | | 5 | | | | 5 |
| 100 | | | | | | | | | | | | | | | | | 1 | | | | 1 |
| 110 | | | | | | | | | | | | | | | | | 1 | | | | 1 |
| 132 | | | | | | | | | | | | | | | | | 2 | | | | 2 |
| 154 | | 1 | | | | | | | | | | | | | | | | | | | 1 |
| 220 | | 1 | | 1 | | | | | | | | | | | | | 1 | | | | 3 |
| An. | | 2 | | | 2 | | | 4 | 2 | | | 1 | | | | 3 | 22 | 1 | | 3 | 39 |
| Total | | 27 | 2 | 25 | 24 | 2 | 8 | 99 | 72 | 7 | 2 | 15 | 5 | 1 | 32 | 8 | 296 | 24 | 5 | 76 | 730 |

to Apocynaceae, Asclepiadaceae, and Loganiaceae than Gentianaceae and Menyanthaceae. The families of Dipsacales are less similar to Rubiaceae in the base numbers, although the Dipsacaceae might be considered to be close (Table 2). It is possible that the Gentianales had a common origin, from the Saxifragales, with Cornales and Dipsacales as suggested by Takhtajan (1969, 1980). Considering the base number, the Gentianales is similar to Cornales. But the possible determination of the Gentianales from the Saxifragales is

Table 2. Predominant base chromosome numbers in major families of Gentianales, Dipsacales, Cornales, and Saxifragales. Underlined number is the most frequent one. Takhtajan's system is followed.

Gentianales: Apocynaceae $x=10, 11$; Asclepiadaceae 11; Gentianaceae 9, 10, 13; Loganiaceae 10, 11; Menyanthaceae 27; Rubiaceae 9, 11.

Dipsacales: Adoxaceae 9; Caprifoliaceae 9; Dipsacaceae 8, 9, 10; Valerianaceae 7, 8.

Cornales: Apiaceae 8, 11; Araliaceae 12; Cornaceae 8, 9, 11; Garryaceae 11; Nyssaceae 11.

Saxifragales: Bruniaceae 8; Cunoniaceae 12, 15, 16; Grossulariaceae 8; Hydrangeaceae 9; Parnassiaceae 9; Pittosporaceae 12.

Table 3. Number of polyploid species of Rubiaceae with different base chromosome numbers.

| x: | Total species | Polyploid species | % of polyploid |
|-------|---------------|------------------------------------|----------------|
| 6 | 5 | 0 | 0.0 |
| 7 | 16 | 8 (4, 8, 12 x) | 50.0 |
| 8 | 9 | 6 (4 x) | 66.7 |
| 9 | 84 | 47 (4, 6, 8 x) | 56.0 |
| 10 | 30 | 15 (4, 10 x) | 50.0 |
| 11 | 493 | 200 (3, 4, 6, 8, 10, 12, 14, 20 x) | 40.6 |
| 12 | 30 | 15 (0) | 50.0 |
| 13 | 6 | 0 | 0.0 |
| 14 | 8 | 0 | 0.0 |
| 17 | 8 | 3 (0) | 37.5 |
| An. | 41 | - | - |
| Total | 730 | 294 (4-20 x) | 40.2 |

not clear. Bentham and Hooker's (1876) and Bensen's (1957) placement of Rubiaceae together with the families of Dipsacales in the order Rubiales is not supported by the evidence obtained from the base numbers. Rubiaceae of the Gentianales as treated by Takhtajan (1980) and Dahlgren (1983) seems to more accurately reflect the chromosome data since majority of the family is $x=11$. It should be noted again that the base number is not enough to discuss about relationships.

Approximately 30-35 % of the species of flowering plants are polyploids (Stebbins 1971). The known taxa of Rubiaceae reveal approximately 40 % polyploidy. The number of polyploid species with different base numbers is presented in Table 3. The percentages may reflect a bias because not enough species has been examined except for $x=11$. The polyploid series of $x=11$ reveal a wide range of repeated polyploidy, from triploidy to 20-ploidy: Ixora rosea Wall. (3-ploidy), Nertera scapanioides Lange (4-ploidy), Galium boreale L. (5-ploidy), Rubia peregrina L. (6-ploidy), Myrmecodia echinata Gaudich (8-ploidy), G. anisophyllum Vill (10-ploidy), Rubia peregrina L. (12-ploidy), Coprosma pumila Hook f. (14-ploidy), and C. ernodeoides

Table 4. Geographical distribution of selected 69 genera of Rubiaceae according to base chromosome number. Number in parentheses indicates polyploid genera. The 69 genera were selected from both well known distribution data and chromosome numbers. Genera including more than one category in either geographical distribution or chromosome numbers are considered duplicately as much as they are frequent.

| x: | area | Tropical Africa | South Africa | Europe | Tropical & subtropical Asia | Australia | Oceania | South America | Central & tropical America | Total |
|-------|------|-----------------|--------------|--------|-----------------------------|-----------|---------|---------------|----------------------------|----------|
| 6 | | 2(1) | - | - | 2(1) | - | - | - | 2(1) | 6(3) |
| 7 | | 6(5) | 2(2) | - | 3(2) | - | - | 2(1) | 6(4) | 19(14) |
| 8 | | 3(1) | - | - | 2(1) | - | - | - | 2(1) | 7(3) |
| 9 | | 10(5) | 1(0) | 1(0) | 5(3) | - | - | 1(1) | 8(7) | 26(16) |
| 10 | | 3(2) | 2(2) | - | 3(2) | 3(3) | 2(2) | 2(2) | 3(2) | 18(15) |
| 11 | | 27(12) | 14(4) | 6(4) | 33(17) | 8(6) | 5(3) | 12(9) | 18(10) | 119(65) |
| 12 | | 2(1) | 1(1) | - | 2(0) | - | - | 2(1) | 3(0) | 10(3) |
| 13 | | - | - | - | - | - | - | 1(0) | 1(0) | 2(0) |
| 14 | | 1(0) | - | - | 1(0) | - | - | - | - | 2(0) |
| 17 | | - | - | - | 1(1) | - | - | - | 2(1) | 3(1) |
| Total | | 57(27) | 16(9) | 7(4) | 52(27) | 11(9) | 7(5) | 20(14) | 45(26) | 212(121) |

A. Gray (20-ploidy).

Polyploidy has been much compounded in the tribes such as Hedyotideae, Ixoreae, and Rubieae; and probably most often been associated with hybridization. It is believed that both polyploidy and aneuploidy in the $x=11$ series seem to have been particularly effective in speciation. It is also noticeable that the most advanced family characteristics can be found in the taxa with repeated polyploidy of $x=11$.

A few epiphytes in the tribe Psychotrieae (Hydnophytum, Myrmecodia etc.) are considered advanced species. Scandent plants occur in the tribes: Ixoreae, Naucleae, Paederieae (mostly), and Vanguerieae. Unisexual flowers occur in a few Gardenieae, very few Rubieae (some Asperula), and many Anthospermeae. Only taxa from one tribe, Guettardeae, have seeds without endosperm. Aggregate fruits are found in Morinda and some Naucleae. Dry fruits occur in a large number of genera (Verdcourt 1958). These examples indicated above occur only in the $x=11$ series, which is considered to be an actively developing group in Rubiaceae. The occurrence of base number $x=11$ in many large and diverse tribes also suggest a complement of some antiquity (Lewis 1962) (Table 1).

In the chromosome number distribution in the family, the diploid numbers in the Group "A" (Table 1) with base number of $x=9$, 10, 11, and 12 reappeared in the Group "B" (Table 1) after chromosome doubling through probably natural hybridization. The Group "B" likely

resulted from tetraploidy of the same base numbers.

Phytogeography sometimes provides information which helps delineate groupings. The geographical distribution of 69 genera with different base numbers are presented in Table 4. The family has a wide distribution and this probably indicates a very ancient family. With predominant pantropical distribution in modern flora, there is little doubt that Rubiaceae had their major evolution in the tropics, possibly beginning in the Triassic or Jaurassic followed by exploitation of some extra-tropical environment in the Cretaceous (Axelrod 1960). Verdcourt (1958) indicated chromosome counts would be of little assistance in classification of taxa in tribes. The tribes widespread throughout tropics, such as Psychotrieae, Morindeae, Gardenieae, Ixoreae, and Paederieae have a $x=11$ base number only. The tropical tribes widely distributed but not in Australia, such as Mussaendeae and Cinchoneae have a base number of $x=9$ through 13 or 17. The tribes occurring in both tropics and temperate regions, such as Hedyotideae, Spermacoceae, Anthospermeae, and Rubieae reveal a diverse base number $x=7$ through 13 or 14. Tropical tribes not represented in Africa, such as Rondeletieae and Chiocceae have a base number of $x=11$ or 12; the one not represented in South America, such as Ophiorrhiza, Urophyllum and Knoxiaceae $x=9$, 10, or 11. In the Rubiaceae, or at least some group of them, the idea that the base number and geographical distribution are correlated may be valuable on the assumption since many tribes having only $x=11$ remain the tropics, and the tribes having diversified base numbers have spread to a wider geographical distribution. It seems possible when consideration is given to diverse characters that the tropical tribes having only $x=11$ are the oldest.

Rubieae is the tribe with apolyploid series of $x=11$ which has been successful in many geographical area. Many of the Rubieae have a large number of different fruit types, which indicate many dispersal adaptation such as: the pericarp being dry or flesh, their surface being covered with hooked or straight hairs, papillae, or wings, or smooth. In derived groups the modification of pedicels, peduncles and bracts had led to elaborate complex dispersal structures.

Polyploids usually have different geographic distribution from their diploid ancestors, and are likely to be particularly frequent and diverse in regions newly opened to colonization (Stebbins 1950). Polyploid elements of the strictly annual and predominantly autogamous Galium aparine complex are among the most successful colonizing weeds of flowering plants and accompanied man to many parts of world. This complex originated by allopolyploidy (2, 4, 6, 8, 10x) from basic diploids with structural and dysploid differentiation of genomes ($x=11$ and 10) (Ehrendorfer 1965).

As many researchers have found, the chromosome numbers of a species can not be doubled indefinitely without deleterious results (Stebbins 1950). For each species, there seems to be an optimum chromosome number, which may be diploid, tetraploid, or hexaploid, but rarely as high as those in Hedyotideae and Rubieae. It seems apparent that additional study of the chromosomes combined with other

available data would be possible to determine the inter-/intra-relationships better.

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